TRADE SECRET

Study Title

H-28548: Inhalation Acute Exposure With Anatomic Pathology Evaluation in Rats

AUTHOR: Thomas A. Kegelman, A.A.

STUDY COMPLETED ON: May 11, 2009

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
DuPont Haskell Global Centers for
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U.S.A.

LABORATORY PROJECT ID: DuPont-17751-723

WORK REQUEST NUMBER: 17751

SERVICE CODE NUMBER: 723

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD and MAFF (Japan) Good Laboratory Practices.

Study Director: Thomas A. Kegelman 11-May-2009

Thomas A. Kegelman, A.A.
Senior Staff Toxicologist

Date

QUALITY ASSURANCE STATEMENT

Work Request Number: 17751
Service Code Number: 723

Key inspections for DuPont work request 17751, service code 723 were completed by the Quality Assurance Unit of DuPont and the findings were submitted on the following dates.

Phase Audited	***Audit Dates***	***Date Reported to Study Director***	***Date Reported to Management***
Protocol:	September 02, 2008	September 03, 2008	September 03, 2008
Conduct:	September 04, 2008	September 04, 2008	September 04, 2008
Report/Records:	May 07,08, 2009	May 08, 2009	May 08, 2009

Reported by: _____ 11May09
Antonio Pedulla Date
Quality Assurance Auditor

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Anatomic Pathology Evaluation by: Steven R. Frame
Steven R. Frame, D.V.M., Ph.D., Diplomate ACVP
Manager
11 May 2009
Date

Approved by: Scott E. Loveless
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David P. Kelly, B.S.
Research Toxicologist
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Issued by Study Director: Thomas A. Kegelman
Thomas A. Kegelman, A.A.
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11-May-2009
Date

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF APPENDICES

Page

STUDY INFORMATION

Substance Tested:
- HFPO Dimer Acid Ammonium Salt
- 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid, ammonium salt
- 62037-80-3 (CAS Number)
- H-28548

Haskell Number: 28548

Composition:

84%	HFPO Dimer Acid Ammonium Salt
12.7%	Water
150 ppm	Perfluorooctanoic acid

Purity: 84% (based on Certificate of Analysis)

Physical Characteristics: Clear and colorless liquid

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Study Initiated/Completed: September 2, 2008 / (see report cover page)

Experimental Start/Termination: September 2, 2008 / May 11, 2009

SUMMARY

One group of 5 male and 5 female rats was exposed to an aerosol atmosphere containing 5200 mg/m³ of H-28548 in air to determine the inhalation median lethal concentration (LC_{50}). Two groups of 3 male and 3 female rats each were exposed to 13 and 100 mg/m³ H-28548 in air to evaluate respiratory tract pathology. As a control for the pathology evaluation, an additional group of one male and one female rat was exposed to air only. All rats were exposed nose-only for a single 4-hour period. Aerosol atmospheres were generated by nebulization, and concentrations of H-28548 were measured by gravimetric analysis. The ammonia vapor concentration was monitored with Draeger tubes. The ammonia concentration measured during the 0 and 13 mg/m³ exposures was less than 1 ppm. During the 100 and 5200 mg/m³ exposures the ammonia concentrations were 21 ppm and 960 ppm, respectively.

Rats in the 0, 13, and 100 mg/m³ exposure groups were weighed and observed for clinical signs of toxicity during a 2-day recovery period. The rats in the 5200 mg/m³ exposure group were weighed and observed for clinical signs of toxicity during a 14-day recovery period. Gross examinations were performed on all rats, and respiratory tract tissues (lung, larynx/pharynx, trachea, and nose) from the 0, 13, and 100 mg/m³ groups were evaluated microscopically. There were no microscopic examinations of rats in the 5200 mg/m³ exposure group.

No deaths occurred in any exposure group. There were no clinical signs of toxicity observed during any exposure in this study. Immediately following the 100 mg/m³ exposure, all rats displayed red nasal discharge. Red discharges around the eyes, nose and mouth were observed in all rats immediately after the 5200 mg/m³ exposure. Rats from the 5200 mg/m³ exposure group also displayed red-stained faces and/or heads for up to 2 days post exposure. Other than the stains and discharges noted immediately after the 100 and 5200 mg/m³ exposures, there were no other toxicologically significant clinical signs, gross pathological or microscopic findings in any rats from any exposure group.

Body weight patterns in the 13 and 100 mg/m³ exposure groups were similar to those exhibited by the control rats. Rats in the 5200 mg/m³ exposure group lost from 2.5 to 6.8% of their original body weight for one or 2 days post exposure, followed by normal weight gain throughout the remainder of the recovery period.

Under the conditions of this study, the 4-hour inhalation median lethal concentration (LC_{50}) for aerosols of H-28548 in male and female rats was greater than 5200 mg/m³. No microscopic changes were observed in the upper or lower respiratory tract at concentrations up to 100 mg/m³, the highest concentration evaluated microscopically.

INTRODUCTION

The objective of this study was to evaluate the 4-hour LC_{50} and acute inhalation toxicity of H-28548. The inhalation route of exposure was chosen based on the expected route of potential human exposure.

ANIMAL WELFARE ACT COMPLIANCE

This study complied with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR) and the Guidelines from the Guide for the Care and Use of Laboratory Animals (NRC 1996). The sponsor should make particular note of the following:

- The signature of the sponsor and/or the study director ensures that the study described in this report does not unnecessarily duplicate previous experiments, and is in compliance with the DuPont Policy on Animal Testing.

- Whenever possible, procedures used in this study have been designed to implement a reduction, replacement, and/or refinement in the use of animals in an effort to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study report or in written laboratory standard operating procedures.

- DuPont Haskell policy is that animals experiencing severe pain or distress that cannot be relieved are painlessly euthanized, as deemed appropriate by the veterinary staff and study director or appropriate designee. The sponsor was advised by the study director of all circumstances that could lead to this action in as timely a manner as possible.

- Methods of euthanasia used during this study were in conformance with the above referenced regulation and the recommendations of the American Veterinary Medical Association (AVMA), 2007 Guidelines on Euthanasia.

- Animals were provided with species-appropriate environmental enrichment.

- DuPont Haskell is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

STUDY DESIGN

One group of 5 male and 5 female rats was exposed to the test substance in air to determine the inhalation median lethal concentration (LC_{50}). Two groups of 3 male and 3 female rats each were exposed to the test substance in air to examine respiratory tract pathology. As a control group for the pathology evaluation, an additional group of one male and one female rat was exposed to air only. Rats were exposed nose-only for a single, 4-hour period.

Following exposure, rats were retained for a recovery period of 2 or 14 days. Rats were approximately 8 weeks old; male rats weighed between 254 and 314 grams, and female rats weighed between 196 and 223 grams at the time of exposure.

Animals were observed for mortality and response to alerting stimuli 3 times during each exposure and observed for mortality and clinical signs of toxicity immediately after they were removed from the restrainers following exposure. During the recovery period, all rats were observed each day for mortality. Rats were weighed and observed for clinical signs of toxicity on the day following exposure and at least twice more during the recovery period. At the end of the recovery period, all rats were sacrificed by carbon dioxide asphyxiation and the designated animals were given anatomic pathology evaluations.

MATERIALS AND METHODS

A. Test Substance

(Appendix A)

The test substance, H-28548, was supplied by the sponsor as a clear and colorless liquid with a purity of 84% by analysis. The test substance was assumed to be stable throughout the exposure phase of the study; no evidence of instability was observed.

B. Test System

Young adult, male and female Crl:CD(SD) rats were received from Charles River Breeding Laboratories, Raleigh, North Carolina. The rats were approximately 7 weeks old on the day of arrival.

Rats have historically been used in safety evaluation studies for acute inhalation toxicity testing. The Crl:CD(SD) rat was selected based on consistently acceptable health status and on extensive experience with the strain at DuPont Haskell.

C. Animal Husbandry

1. Quarantine

Animals were quarantined after arrival for 6 days prior to testing. During the quarantine period, animals were weighed and observed for clinical signs of disease.

2. Animal Selection

Prior to each exposure, male and female rats were selected for use on the study from the rats that were released from quarantine, had been gaining weight at a normal rate, had no overt signs of disease, and were the appropriate age and body weight. No attempt was made to randomly group animals.

3. Identification

Each animal was assigned an animal number that was recorded on a card affixed to the cage. Prior to exposure, the tail of each animal and cage card were coded with water-insoluble pens so that each animal could be identified after exposure and during the recovery period.

4. Housing

Except during exposure, animals were housed individually in solid bottom caging with bedding.

5. Environmental Conditions

Animals were housed in proximity to the inhalation chambers. Animal rooms were maintained at a temperature of 18-26ºC and a relative humidity of 30-70%. Animal rooms were artificially illuminated (fluorescent light) on an approximate 12-hour light/dark cycle. Any excursions outside of these ranges were of insufficient magnitude and/or duration to have adversely affected the validity of the study.

6. Feed and Water

Except during exposure, PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 and tap water were available *ad libitum*.

7. Animal Health and Environmental Monitoring Program

As specified in the DuPont Haskell animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.

- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Evaluation of these data did not indicate any conditions that affected the validity of the study.

D. Duration of Exposure

Each group of animals was exposed for 4 hours. The starting time of each exposure was defined as the time when the generation system was turned on. The ending time of each exposure was defined as the time when the generation system was turned off. Animals were exposed to the test substance during both the time it took for the chamber to reach concentration, and the time it took for the test substance to be purged from the chamber. After each exposure, animals were returned to their cages. The end of the recovery period for the 0, 13 and 100 mg/m³ exposure group was defined as the second day after the exposure and the 14th day after exposure for the 5200 mg/m³ exposure group.

E. Inhalation Exposure System

(Figure 1)

1. Atmosphere Generation

Chamber atmospheres were generated by nebulization of the test substance in air with a Spraying Systems nebulizer. The test substance was metered into the nebulizer with a Harvard Apparatus model 22 syringe infusion pump. High-pressure air, metered into the nebulizer by a Brooks model 5850E mass flow controller, carried the resulting atmosphere into the exposure chamber. To attain the 13 mg/m³ chamber aerosol concentration, the nebulizer sprayed into a glass cyclone to reduce the amount of aerosol particles entering the exposure chamber. The infusion pump and mass flow controller were controlled and monitored by the Camile Inhalation Toxicology Automated Data System (CITADS). Chamber concentrations of test substance were controlled by varying the test substance feed rate or airflow to the nebulizer.

Test atmospheres were exhausted through a scrubber with water followed by an MSA charcoal/HEPA filter cartridge prior to discharge into the fume hood.

2. Chamber Construction and Design

The exposure chamber was constructed of glass (cylindrical) with a nominal internal volume of 34 L. A baffle inside the chamber promoted uniform chamber distribution of the test atmosphere.

3. Exposure Mode

During exposure, animals were individually restrained in perforated stainless steel cylinders with conical nose pieces. The restrainers were inserted into a polymethylmethacrylate faceplate attached to the exposure chamber so that the nose of each animal extended into the exposure chamber.

F. Characterization of Chamber Atmosphere

1. Test Substance Sampling and Analysis

During each exposure, the atmospheric concentration of the test substance was determined by gravimetric analysis at least 4 times in the test chambers and once during the air-only exposure. Known volumes of chamber atmosphere were drawn from the sampling port through a 25 mm filter cassette containing a pre-weighed glass fiber (Type A/E) filter. The filters were weighed on a Cahn model C-30 Microbalance®. The filter weights were automatically transferred to the CITADS, which calculated the chamber concentrations based on the difference between pre- and post-sampling filter weights divided by the volume of chamber atmosphere sampled. Gravimetric sample start- and stop-times for each sample were controlled and recorded by CITADS.

Upon completion of the exposures, CITADS sample results were transferred to the Camile Inhalation Reporting and Analysis System (CIRAS), which collates sample calculations.

2. Particle Size Determination

A sample to determine particle size distribution (mass median aerodynamic diameter, geometric standard deviation, and percent particles less than 1, 3, and 10 µm diameter) was taken during the 13, 100, and 5200 mg/m³ exposures with a Sierra® series 210 cyclone preseparator/cascade impactor and Sierra® series 110 constant flow air sampler.[(1)]

3. Environmental Monitoring

Chamber temperature was targeted at 20-24°C. The temperature was monitored continually with a traceable thermocouple thermometer and recorded approximately every 30 minutes during each exposure. Chamber relative humidity was targeted at 30-70%. The relative humidity was measured with an Omega model RH5100 digital psychrometer and recorded 3 times during each exposure. Chamber airflow was set at the beginning of each exposure to achieve at least 10 air changes per hour. The airflow was monitored continually with a Brooks model 5850E mass flow controller and recorded approximately every 30 minutes during each exposure. Chamber oxygen concentration was targeted to be at least 19%. The oxygen concentration was measured with a Biosystems model 3100R oxygen analyzer and recorded 3 times during each exposure.

G. Anatomic Pathology Evaluation

Rats from the 0, 13, and 100 mg/m³ exposures were sacrificed for anatomic pathology evaluation. On the last day of the recovery period, all rats were euthanized by carbon dioxide asphyxiation and exsanguination.

Gross examinations were performed on all rats. The lung, larynx/pharynx, trachea, and nose of rats in the 0, 13, and 100 mg/m³ exposure groups were collected and fixed in 10% neutral buffered formalin. Tissues were processed and embedded in paraffin, sectioned approximately 5-6 microns thick, stained with hematoxylin and eosin (H&E), and examined microscopically.

RESULTS AND DISCUSSION

Inhalation Exposures

A. Exposure Conditions

(Tables 1-2)

1. Chamber Distribution of Aerosol

To determine the chamber distribution of aerosol particles, a trial generation was conducted without animals using the same generation parameters that were used to generate the 5200 mg/m³ chamber atmosphere. Gravimetric chamber atmosphere samples were collected from 3 separate locations in the faceplate and at the reference port of the exposure chamber, averaged, and individual samples from the faceplate compared to the overall average. Samples taken from the faceplate demonstrated differences that were less than 10% (maximum difference 6.8%) from the overall mean aerosol concentration. Since the difference was less than 10%, the aerosol was considered to be homogeneously distributed at the breathing zone of the animals. Therefore, the use of the reference port for air sampling was considered adequate.

2. Mean Concentration and Particle Size Distribution

Animals were exposed to H-28548 at mean total concentrations of 0, 13, 100 or 5200 mg/m³. Corresponding ammonia vapor concentrations were less than 1 ppm during the 0 and 13 mg/m³ exposures and 21 and 960 ppm during the 100 and 5200 mg/m³ exposure, respectively. The mass median aerodynamic diameters (MMAD) measured for the H-28548 atmospheres ranged from 1.7 to 2.7 µm with geometric standard deviations ranging from 2.1 to 2.7. Therefore, atmospheres generated in this study were considered respirable in rats.

3. Chamber Environmental Conditions

Chamber temperatures ranged from 19 to 22°C, chamber relative humidity ranged from 43 to 60%, chamber airflow was 14 L/minute, and the chamber oxygen concentration was 21%. Although the chamber temperature was slightly lower than the targeted parameter during the 5200 mg/m³ exposure, the chamber environmental conditions were considered adequate for this study.

In-Life Measurements

A. Body Weights and Clinical Observations

(Tables 3-5, Appendices B-C)

Some minor body weight losses were observed in male and female rats in the 0, 13 and 100 mg/m³ exposure groups. Since the body weight losses in the 13 and 100 mg/m³ exposure groups were similar to that of the control rats, these body weight losses are not considered

toxicologically significant. Rats in the 5200 mg/m³ exposure group lost from 2.5 to 6.8% of their original body weight for one or 2 days post exposure.

There were no clinical signs of toxicity observed during any exposure in this study. Immediately following the 100 mg/m³ exposure, all rats displayed red nasal discharge. Red discharge around the eyes, nose and mouth were observed in all rats immediately after the 5200 mg/m³ exposure. Rats from the 5200 mg/m³ exposure group also displayed red stained faces and/or heads for up to 2 days post exposure. The clinical signs observed immediately after the exposure and the red stained faces and heads were the only clinical signs of toxicity noted in this study.

Anatomic Pathology Evaluation

A. Cause of Death

There were no deaths attributed to the test substance **in this study.**

B. Gross Observations

(Tables 6-9, Appendix D)

There were no gross observations noted during necropsy.

C. Microscopic Findings

(Tables 10-11, Appendix D)

There were no test substance-related microscopic findings in rats in the 0, 13 and 100 mg/m³ exposure groups. The 5200 mg/m³ exposure was conducted for determining an LC_{50} value and therefore organs or tissues from rats in this exposure group were not examined microscopically.

CONCLUSIONS

Under the conditions of this study, the 4-hour inhalation median lethal concentration (LC_{50}) for aerosols of H-28548 in male and female rats was greater than 5200 mg/m³. No microscopic changes were observed in the upper or lower respiratory tract at concentrations up to 100 mg/m³, the highest concentration evaluated microscopically.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at DuPont Haskell, Newark, Delaware, Iron Mountain Records Management, Wilmington, Delaware, or Quality Associates Incorporated, Fulton, Maryland.

REFERENCES

1. Calculation described in Sierra Instruments, Inc., Bulletin 7-79-219IM, Instruction Manual: Series 210 Ambient Cascade Impactors and Cyclone Preseparators.

TABLES

TABLES

EXPLANATORY NOTES

ABBREVIATIONS:

Chamber Concentrations of H-28548 and Particle Size Distribution

S.D. - standard deviation
mg/m^3 - milligram per cubic meter
n - number of samples

NOTES:

Chamber Concentrations of H-28548 and Particle Size Distribution
Chamber Environmental Conditions
Values are reported to 2 significant figures.
Calculations were performed prior to rounding values.

Table 1
Chamber Concentrations of H-28548 and Particle Size Distribution

ATMOSPHERIC CONCENTRATION (mg/m³)				MASS MEDIAN AERODYNAMIC DIAMETER (µm)[a]	GEOMETRIC STANDARD DEVIATION	% PARTICLES BY MASS		
MEAN	S.D.	RANGE	n			<1 µm	<3 µm	<10 µm
0	-	-	1	-	-	-	-	-
13	3.4	9.5 - 17	4	2.2	2.1	15	67	98
100	24	77 - 140	5	1.7	2.4	28	75	98
5200	2000	3600 - 8600	5	2.7	2.7	16	55	91

a One sample taken during each exposure.

Table 2
Chamber Environmental Conditions

MEAN ATMOSPHERIC CONCENTRATION (mg/m³)	TEMPERATURE RANGE (°C)	RELATIVE HUMIDITY RANGE (%)	AIRFLOW RANGE (L/min)
0	21	48 - 52	14
13	21	50 - 54	14
100	21 - 22	43 - 48	14
5200	19	56 - 60	14

Table 3
Mortality

SEX	MEAN ATMOSPHERIC CONCENTRATION (mg/m³)	MORTALITY (deaths / exposed)
MALE	0	0 / 1
	13	0 / 3
	100	0 / 3
	5200	0 / 5
FEMALE	0	0 / 1
	13	0 / 3
	100	0 / 3
	5200	0 / 5

Table 4
Summary of Clinical Observations in Male Rats

	Day numbers relative to Start Date			
	Group 1	Group 2	Group 3	Group 4
Concentration (mg/m³)	0	13	100	5200
Animal Count	1	3	3	5
Discharge - red				
Number of Observations	.	.	3	5
Number of Animals	.	.	3	5
Days from - to	.	.	0 0	0 0
Stained skin/fur - red				
Number of Observations	.	.	.	6
Number of Animals	.	.	.	5
Days from - to	.	.	.	1 2

Table 5
Summary of Clinical Observations in Female Rats

	Day numbers relative to Start Date			
	Group 1	Group 2	Group 3	Group 4
Concentration (mg/m³)	0	13	100	5200
Animal Count	1	3	3	5
Discharge - red				
Number of Observations	.	.	3	5
Number of Animals	.	.	3	5
Days from - to	.	.	0 0	0 0
Stained skin/fur - red				
Number of Observations	.	.	.	6
Number of Animals	.	.	.	5
Days from - to	.	.	.	1 2

Table 6
Incidence of Gross Observations in Male Rats
Test Day 2 Sacrifice

Group:	1	2	3
Concentration (mg/m³):	0	13	100
Number of Animals on Study :	1	3	3

No gross lesions were present in the rats at necropsy.

Table 7
Incidence of Gross Observations in Male Rats
Test Day 14 Sacrifice

Group:	4
Concentration (mg/m³):	5200
Number of Animals on Study :	5

No gross lesions were present in the rats at necropsy.

Table 8
Incidence of Gross Observations in Female Rats
Test Day 2 Sacrifice

Group:	1	2	3
Concentration (mg/m³):	0	13	100
Number of Animals on Study :	1	3	3

No gross lesions were present in the rats at necropsy.

Table 9
Incidence of Gross Observations in Female Rats
Test Day 14 Sacrifice

Group:	4
Concentration (mg/m³):	5200
Number of Animals on Study :	5

No gross lesions were present in the rats at necropsy.

Table 10
Incidences and Lesion Grades of Microscopic Findings in Male Rats
Test Day 2 Sacrifice

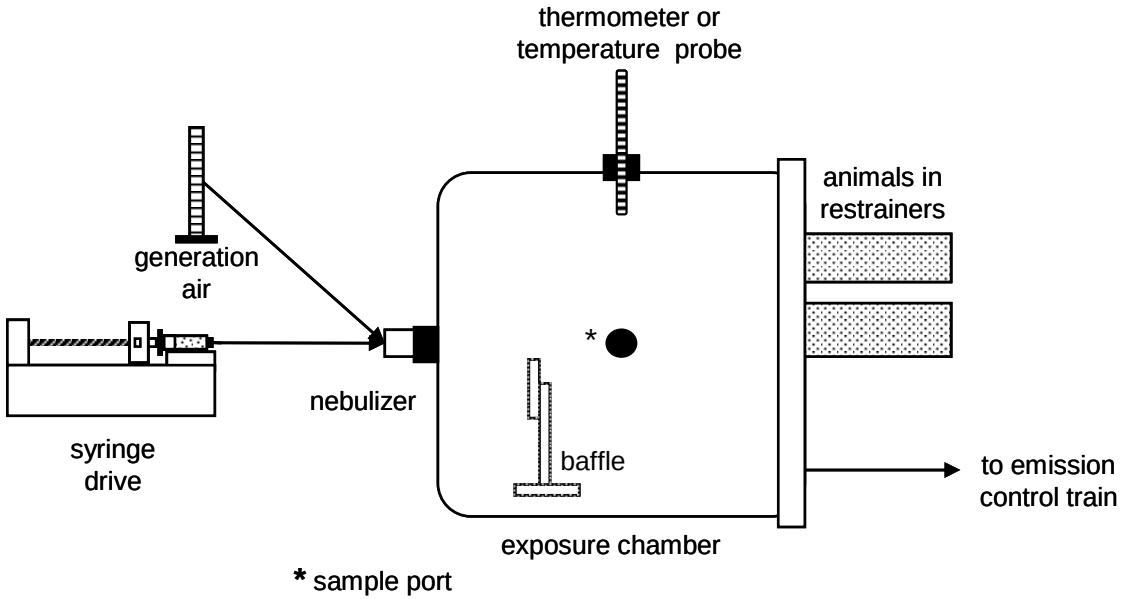
Group:	1	2	3
Concentration (mg/m³):	0	13	100
Number of Animals on Study :	1	3	3
LUNGS;			
Examined	(1)	(3)	(3)
Within Normal Limits	1	3	3
PHARYNX/LARYNX;			
Examined	(1)	(3)	(3)
Within Normal Limits	1	3	3
TRACHEA;			
Examined	(1)	(3)	(3)
Within Normal Limits	1	3	3
NOSE;			
Examined	(1)	(3)	(3)
Within Normal Limits	1	3	2
Inflammation; subacute/chronic; subcutaneous	(0)	(0)	(1)
minimal	0	0	1

Table 11
Incidences and Lesion Grades of Microscopic Findings in Female Rats
Test Day 2 Sacrifice

Group:	1	2	3
Concentration (mg/m³):	0	13	100
Number of Animals on Study :	1	3	3
LUNGS;			
Examined	(1)	(3)	(3)
Within Normal Limits	0	3	3
Inflammation; perivascular/peribronchiolar	(1)	(0)	(0)
minimal	1	0	0
PHARYNX/LARYNX;			
Examined	(1)	(3)	(3)
Within Normal Limits	1	3	2
Inflammation; foreign body	(0)	(0)	(1)
mild	0	0	1
TRACHEA;			
Examined	(1)	(3)	(3)
Within Normal Limits	1	3	3
NOSE;			
Examined	(1)	(3)	(3)
Within Normal Limits	1	3	3

FIGURE

Figure 1
Schematic of Exposure System



Note: The control chamber set-up did not include syringe drive. The 13 mg/m³ chamber set-up employed a 2-L glass cyclone before the nebulizer.

APPENDICES

Appendix A
Certificate of Analysis

E. I. du Pont de Nemours and Company
Wilmington, DE 19898
USA

CERTIFICATE OF ANALYSIS

This Certificate of Analysis fulfills the requirement for characterization of a test substance prior to a study subject to GLP regulations. It documents the identity and content of the test substance. This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 792).

Haskell Code Number	H-28548
Common Name	HFPO Dimer Acid Ammonium Salt
Purity Percent	84%
Other Components	Water – 12.7% Perfluorooctanoic acid – 150 ppm
Date of Analysis	June 13, 2008
Recommended reanalysis interval	1 year
Instructions for storage	NRT&H
Reference	DuPont-25455
Analysis performed at	E. I. DuPont de Nemours and Company DuPont Haskell Laboratories Newark, Delaware USA

Approver:

Peter A. Bloxham, Ph.D.
Senior Research Chemist

18-JUNE-2008
Date

Appendix B
Individual Body Weights

INDIVIDUAL BODY WEIGHTS

EXPLANATORY NOTES

ABBREVIATIONS:

g - gram

Individual Body Weights

Group Sex	Animal Number	Bodyweight (g) Day numbers relative to Start Date 0	1	2	3	7	14
1m	101	291.2	285.5	296.5	.	.	.
2m	201	286.6	285.4	293.3	.	.	.
	202	301.3	299.9	315.1	.	.	.
	203	272.6	279.0	290.0	.	.	.
3m	301	280.0	281.8	290.9	.	.	.
	302	254.0	253.9	261.5	.	.	.
	303	279.0	275.8	286.3	.	.	.
4m	401	285.0	267.9	273.3	282.3	311.1	335.7
	402	302.5	281.9	288.0	301.5	338.9	370.8
	403	289.0	270.9	271.7	279.9	310.8	346.1
	404	290.6	273.4	277.3	291.9	329.7	361.3
	405	313.8	300.8	297.6	313.8	356.8	401.5

Nominal Dose: Group 1 - 0 mg/m³ Group 2 - 13 mg/m³ Group 3 - 100 mg/m³ Group 4 - 5200 mg/m³

Individual Body Weights

Group Sex	Animal Number	Bodyweight (g) Day numbers relative to Start Date 0	1	2	3	7	14
1f	151	195.8	195.0	201.9	.	.	.
2f	251	197.1	203.4	210.3	.	.	.
	252	203.8	200.8	203.5	.	.	.
	253	196.2	201.6	206.5	.	.	.
3f	351	217.0	211.5	215.6	.	.	.
	352	200.0	195.4	202.5	.	.	.
	353	204.0	206.7	208.8	.	.	.
4f	451	223.3	216.4	217.3	221.1	247.7	265.3
	452	218.9	204.4	209.0	210.9	233.4	243.3
	453	215.7	206.4	206.6	212.1	219.4	239.6
	454	209.9	204.6	206.5	208.2	229.7	251.5
	455	205.6	206.6	199.2	210.1	225.6	233.3

Nominal Dose: Group 1 - 0 mg/m³ Group 2 - 13 mg/m³ Group 3 - 100 mg/m³ Group 4 - 5200 mg/m³

Appendix C
Individual Clinical Observations and Mortality Records

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS

EXPLANATORY NOTES

ABBREVIATIONS:

X - present

Individual Clinical Observations and Mortality Records

Group Sex	Animal Number	Clinical Sign	Site	Day numbers relative to Start Date 0	1	2	3	7	14
1m	101	No Abnormalities Detected		X	X	X	.	.	.
		Scheduled sacrifice		.	.	X	.	.	.
2m	201	No Abnormalities Detected		X	X	X	.	.	.
		Scheduled sacrifice		.	.	X	.	.	.
	202	No Abnormalities Detected		X	X	X	.	.	.
		Scheduled sacrifice		.	.	X	.	.	.
	203	No Abnormalities Detected		X	X	X	.	.	.
		Scheduled sacrifice		.	.	X	.	.	.
3m	301	No Abnormalities Detected		.	X	X	.	.	.
		Discharge - red	Nose	X	.	.	.	.	.
		Scheduled sacrifice		.	.	X	.	.	.
	302	No Abnormalities Detected		.	X	X	.	.	.
		Discharge - red	Nose	X	.	.	.	.	.
		Scheduled sacrifice		.	.	X	.	.	.
	303	No Abnormalities Detected		.	X	X	.	.	.
		Discharge - red	Nose	X	.	.	.	.	.
		Scheduled sacrifice		.	.	X	.	.	.

Nominal Dose: Group 1 - 0 mg/m³ Group 2 - 13 mg/m³ Group 3 - 100 mg/m³ Group 4 - 5200 mg/m³

Individual Clinical Observations and Mortality Records

Day numbers relative to Start Date

Group Sex	Animal Number	Clinical Sign	Site	0	1	2	3	7	14
4m	401	No Abnormalities Detected		.	.	.	X	X	X
		Discharge - red	Eye bilateral	X	.	.	.	.	.
		Discharge - red	Mouth	X	.	.	.	.	.
		Discharge - red	Nose	X	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	X
		Stained skin/fur - red	Face	.	X	X	.	.	.
	402	No Abnormalities Detected		.	.	X	X	X	X
		Discharge - red	Eye bilateral	X	.	.	.	.	.
		Discharge - red	Mouth	X	.	.	.	.	.
		Discharge - red	Nose	X	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	X
		Stained skin/fur - red	Face	.	X	.	.	.	.
	403	No Abnormalities Detected		.	.	X	X	X	X
		Discharge - red	Eye bilateral	X	.	.	.	.	.
		Discharge - red	Mouth	X	.	.	.	.	.
		Discharge - red	Nose	X	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	X
		Stained skin/fur - red	Face	.	X	.	.	.	.
	404	No Abnormalities Detected		.	.	X	X	X	X
		Discharge - red	Eye bilateral	X	.	.	.	.	.
		Discharge - red	Mouth	X	.	.	.	.	.
		Discharge - red	Nose	X	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	X
		Stained skin/fur - red	Face	.	X	.	.	.	.
	405	No Abnormalities Detected		.	.	X	X	X	X
		Discharge - red	Eye bilateral	X	.	.	.	.	.
		Discharge - red	Mouth	X	.	.	.	.	.
		Discharge - red	Nose	X	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	X
		Stained skin/fur - red	Face	.	X	.	.	.	.
		Stained skin/fur - red	Head	.	X	.	.	.	.

Nominal Dose: Group 1 - 0 mg/m³ Group 2 - 13 mg/m³ Group 3 - 100 mg/m³ Group 4 - 5200 mg/m³

Individual Clinical Observations and Mortality Records

Group Sex	Animal Number	Clinical Sign	Site	Day numbers relative to Start Date 0	1	2	3	7	14
1f	151	No Abnormalities Detected		X	X	X	.	.	.
		Scheduled sacrifice		.	.	X	.	.	.
2f	251	No Abnormalities Detected		X	X	X	.	.	.
		Scheduled sacrifice		.	.	X	.	.	.
	252	No Abnormalities Detected		X	X	X	.	.	.
		Scheduled sacrifice		.	.	X	.	.	.
	253	No Abnormalities Detected		X	X	X	.	.	.
		Scheduled sacrifice		.	.	X	.	.	.
3f	351	No Abnormalities Detected		.	X	X	.	.	.
		Discharge - red	Nose	X	.	.	.	.	.
		Scheduled sacrifice		.	.	X	.	.	.
	352	No Abnormalities Detected		.	X	X	.	.	.
		Discharge - red	Nose	X	.	.	.	.	.
		Scheduled sacrifice		.	.	X	.	.	.
	353	No Abnormalities Detected		.	X	X	.	.	.
		Discharge - red	Nose	X	.	.	.	.	.
		Scheduled sacrifice		.	.	X	.	.	.

Nominal Dose: Group 1 - 0 mg/m³ Group 2 - 13 mg/m³ Group 3 - 100 mg/m³ Group 4 - 5200 mg/m³

Individual Clinical Observations and Mortality Records

Day numbers relative to Start Date

Group Sex	Animal Number	Clinical Sign	Site	0	1	2	3	7	14
4f	451	No Abnormalities Detected		.	.	.	X	X	X
		Discharge - red	Eye bilateral	X	.	.	.	.	.
		Discharge - red	Mouth	X	.	.	.	.	.
		Discharge - red	Nose	X	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	X
		Stained skin/fur - red	Face	.	X	X	.	.	.
	452	No Abnormalities Detected		.	.	X	X	X	X
		Discharge - red	Eye bilateral	X	.	.	.	.	.
		Discharge - red	Mouth	X	.	.	.	.	.
		Discharge - red	Nose	X	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	X
		Stained skin/fur - red	Face	.	X	.	.	.	.
	453	No Abnormalities Detected		.	.	X	X	X	X
		Discharge - red	Eye bilateral	X	.	.	.	.	.
		Discharge - red	Mouth	X	.	.	.	.	.
		Discharge - red	Nose	X	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	X
		Stained skin/fur - red	Face	.	X	.	.	.	.
		Stained skin/fur - red	Head	.	X	.	.	.	.
	454	No Abnormalities Detected		.	.	X	X	X	X
		Discharge - red	Eye bilateral	X	.	.	.	.	.
		Discharge - red	Mouth	X	.	.	.	.	.
		Discharge - red	Nose	X	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	X
		Stained skin/fur - red	Face	.	X	.	.	.	.
	455	No Abnormalities Detected		.	.	X	X	X	X
		Discharge - red	Eye bilateral	X	.	.	.	.	.
		Discharge - red	Mouth	X	.	.	.	.	.
		Discharge - red	Nose	X	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	X
		Stained skin/fur - red	Face	.	X	.	.	.	.

Nominal Dose: Group 1 - 0 mg/m³ Group 2 - 13 mg/m³ Group 3 - 100 mg/m³ Group 4 - 5200 mg/m³

Appendix D
Individual Animal Pathology Data

INDIVIDUAL ANIMAL PATHOLOGY DATA

KEY TO APPENDIX

LESION GRADING:

Histopathology changes are described according to their morphologic character, distribution and severity. The distribution (extent of tissue involvement) is indicated, where appropriate, by modifiers such as focal, multifocal, diffuse, unilateral, bilateral, etc. A severity score, if appropriate, is also assigned as follows:

MINIMAL: The amount of change present barely exceeds that which is considered to be within normal limits.

MILD: In general, the lesion is easily identified but of limited severity. The lesion probably does not produce any functional impairment.

MODERATE: The lesion is prominent but there is significant potential for increased severity. Limited tissue or organ dysfunction is possible.

SEVERE: The degree of change is either as complete as considered possible or great enough in intensity or extent to expect significant tissue or organ dysfunction.

COMMENT:

Grades minimal through severe represent progressive involvement/severity along a continuum with minimal lesions being the least severe and severe lesions being the most severe. While the grades refer to the morphologic characteristics of lesions, they also indicate their relative biologic significance.

Gross observations listing multiple masses for a tissue are distinguished with letters (i.e., a, b, c, d, etc.).

Individual Animal Pathology Data

Animal Ref.: 101 Group: 1 Sex: Male Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 0 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/05/08 Study Day No. (Week): 2 (0) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/05/08

Terminal Body Weight: 296.5g

Gross Pathology Observations: None

Histo Pathology Observations: None

The following tissues were within normal limits:

LUNGS	PHARYNX/LARYNX	TRACHEA	NOSE

Individual Animal Pathology Data

Animal Ref.: 201 Group: 2 Sex: Male Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 13 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/05/08 Study Day No. (Week): 2 (0) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/05/08

Terminal Body Weight: 293.3g

Gross Pathology Observations: None

Histo Pathology Observations: None

The following tissues were within normal limits:

LUNGS PHARYNX/LARYNX TRACHEA NOSE

Individual Animal Pathology Data

Animal Ref.: 202 Group: 2 Sex: Male Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 13 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/05/08 Study Day No. (Week): 2 (0) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/05/08

Terminal Body Weight: 315.1g

Gross Pathology Observations: None

Histo Pathology Observations: None

The following tissues were within normal limits:

LUNGS	PHARYNX/LARYNX	TRACHEA	NOSE

Individual Animal Pathology Data

Animal Ref.: 203 Group: 2 Sex: Male Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 13 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/05/08 Study Day No. (Week): 2 (0) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/05/08

Terminal Body Weight: 290g

Gross Pathology Observations: None

Histo Pathology Observations: None

The following tissues were within normal limits:

LUNGS	PHARYNX/LARYNX	TRACHEA	NOSE

Individual Animal Pathology Data

Animal Ref.: 301 Group: 3 Sex: Male Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 100 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/04/08 Study Day No. (Week): 2 (0) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/04/08

Terminal Body Weight: 290.9g

Gross Pathology Observations: None

Histo Pathology Observations: None

The following tissues were within normal limits:

LUNGS	PHARYNX/LARYNX	TRACHEA	NOSE

Individual Animal Pathology Data

Animal Ref.: 302 Group: 3 Sex: Male Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 100 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/04/08 Study Day No. (Week): 2 (0) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/04/08

Terminal Body Weight: 261.5g

Gross Pathology Observations: None

Histo Pathology Observations:

NOSE;
Inflammation; subcutaneous; subacute/chronic; minimal

The following tissues were within normal limits:

LUNGS	PHARYNX/LARYNX	TRACHEA

Individual Animal Pathology Data

Animal Ref.: 303 Group: 3 Sex: Male Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 100 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/04/08 Study Day No. (Week): 2 (0) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/04/08

Terminal Body Weight: 286.3g

Gross Pathology Observations: None

Histo Pathology Observations: None

The following tissues were within normal limits:

LUNGS	PHARYNX/LARYNX	TRACHEA	NOSE

Individual Animal Pathology Data

Animal Ref.: 401 Group: 4 Sex: Male Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 5200 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/18/08 Study Day No. (Week): 14 (2) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/18/08

Terminal Body Weight: 335.7g

Gross Pathology Observations: None

Individual Animal Pathology Data

Animal Ref.: 402 Group: 4 Sex: Male Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 5200 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/18/08 Study Day No. (Week): 14 (2) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/18/08

Terminal Body Weight: 370.8g

Gross Pathology Observations: None

Individual Animal Pathology Data

Animal Ref.: 403 Group: 4 Sex: Male Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 5200 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/18/08 Study Day No. (Week): 14 (2) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/18/08

Terminal Body Weight: 346.1g

Gross Pathology Observations: None

Individual Animal Pathology Data

Animal Ref.: 404 Group: 4 Sex: Male Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 5200 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/18/08 Study Day No. (Week): 14 (2) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/18/08

Terminal Body Weight: 361.3g

Gross Pathology Observations: None

Individual Animal Pathology Data

Animal Ref.: 405 Group: 4 Sex: Male Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 5200 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/18/08 Study Day No. (Week): 14 (2) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/18/08

Terminal Body Weight: 401.5g

Gross Pathology Observations: None

Individual Animal Pathology Data

Animal Ref.: 151 Group: 1 Sex: Female Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 0 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/05/08 Study Day No. (Week): 2 (0) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/05/08

Terminal Body Weight: 201.9g

Gross Pathology Observations: None

Histo Pathology Observations:

LUNGS;
Inflammation; perivascular/peribronchiolar; minimal

The following tissues were within normal limits:

PHARYNX/LARYNX TRACHEA NOSE

Individual Animal Pathology Data

Animal Ref.: 251 Group: 2 Sex: Female Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 10 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/05/08 Study Day No. (Week): 2 (0) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/05/08

Terminal Body Weight: 210.3g

Gross Pathology Observations: None

Histo Pathology Observations: None

The following tissues were within normal limits:

LUNGS	PHARYNX/LARYNX	TRACHEA	NOSE

Individual Animal Pathology Data

Animal Ref.: 252 Group: 2 Sex: Female Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 10 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/05/08 Study Day No. (Week): 2 (0) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/05/08

Terminal Body Weight: 203.5g

Gross Pathology Observations: None

Histo Pathology Observations: None

The following tissues were within normal limits:

LUNGS	PHARYNX/LARYNX	TRACHEA	NOSE

Individual Animal Pathology Data

Animal Ref.: 253 Group: 2 Sex: Female Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 10 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/05/08 Study Day No. (Week): 2 (0) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/05/08

Terminal Body Weight: 206.5g

Gross Pathology Observations: None

Histo Pathology Observations: None

The following tissues were within normal limits:

LUNGS	PHARYNX/LARYNX	TRACHEA	NOSE

Individual Animal Pathology Data

Animal Ref.: 351 Group: 3 Sex: Female Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 100 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/04/08 Study Day No. (Week): 2 (0) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/04/08

Terminal Body Weight: 215.6g

Gross Pathology Observations: None

Histo Pathology Observations: None

The following tissues were within normal limits:

LUNGS	PHARYNX/LARYNX	TRACHEA	NOSE

Individual Animal Pathology Data

Animal Ref.: 352 Group: 3 Sex: Female Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 100 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/04/08 Study Day No. (Week): 2 (0) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/04/08

Terminal Body Weight: 202.5g

Gross Pathology Observations: None

Histo Pathology Observations: None

The following tissues were within normal limits:

LUNGS PHARYNX/LARYNX TRACHEA NOSE

Individual Animal Pathology Data

Animal Ref.: 353 Group: 3 Sex: Female Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 100 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/04/08 Study Day No. (Week): 2 (0) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/04/08

Terminal Body Weight: 208.8g

Gross Pathology Observations: None

Histo Pathology Observations:

PHARYNX/LARYNX;
Inflammation; foreign body; mild

The following tissues were within normal limits:

LUNGS	TRACHEA	NOSE

Individual Animal Pathology Data

Animal Ref.: 451 Group: 4 Sex: Female Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 5200 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/18/08 Study Day No. (Week): 14 (2) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/18/08

Terminal Body Weight: 265.3g

Gross Pathology Observations: None

Individual Animal Pathology Data

Animal Ref.: 452 Group: 4 Sex: Female Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 5200 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/18/08 Study Day No. (Week): 14 (2) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/18/08

Terminal Body Weight: 243.3g

Gross Pathology Observations: None

Individual Animal Pathology Data

Animal Ref.: 453 Group: 4 Sex: Female Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 5200 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/18/08 Study Day No. (Week): 14 (2) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/18/08

Terminal Body Weight: 239.6g

Gross Pathology Observations: None

Individual Animal Pathology Data

Animal Ref.: 454 Group: 4 Sex: Female Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 5200 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/18/08 Study Day No. (Week): 14 (2) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/18/08

Terminal Body Weight: 251.5g

Gross Pathology Observations: None

Individual Animal Pathology Data

Animal Ref.: 455 Group: 4 Sex: Female Species: Rat Strain: Crl:CD(SD)

Test Material: H-28548 Dose: 5200 mg/m³ Route: Inhalation Study Type: Acute
Date of Death : 09/18/08 Study Day No. (Week): 14 (2) Mode of Death: SACRIFICED BY DESIGN
Date of Necropsy: 09/18/08

Terminal Body Weight: 233.3g

Gross Pathology Observations: None